A Correlative Biomarker Analysis of the Combination of Bevacizumab and Carboplatin-Based Chemotherapy for Advanced Nonsquamous Non–Small-Cell Lung Cancer

Results of the Phase II Randomized ABIGAIL Study (BO21015)

Tony Mok, MD, Vera Gorbunova, MD,† Erzsebet Juhasz, MD,‡ Barna Szima, MD,§ Olga Burdaeva, MD,‖ Sergey Orlov, MD, PhD,‖ Chong-Jen Yu, MD, PhD,¶ Venice Archer, MD,# Magalie Hilton, MSc,** Paul Delmar, PhD,** Celine Pallaud, PhD,** and Martin Reck, MD, PhD††*

Introduction: Avastin Biomarkers In lunG And 3D Innovative anaLysis (ABIGAIL), which is a phase II, open-label, randomized study, investigated correlations between biomarkers and best overall response to bevacizumab plus platinum-doublet chemotherapy for patients with advanced/recurrent non–small-cell lung cancer.

Methods: Patients received bevacizumab (7.5 or 15 mg/kg, 3-weekly until disease progression/unacceptable toxicity) plus carboplatin/gemcitabine or carboplatin/paclitaxel (maximum six cycles). Plasma samples (baseline/throughout treatment) were analyzed for vascular endothelial growth factor (VEGF)-A (baseline only), VEGF receptors (VEGFR-1/VEGFR-2), basic fibroblast growth factor, E-selectin, intercellular adhesion molecule-1, and placental growth factor (baseline only). Tumor samples (primary specimen) were analyzed for VEGF-A, VEGFR-1/VEGFR-2, neuropilin (NRP), and CD31. Response was evaluated at baseline and every 6 weeks (Response Evaluation Criteria in Solid Tumors).

Results: Patients were randomized to receive chemotherapy plus 7.5 mg/kg (n = 154) or 15 mg/kg (n = 149) bevacizumab. For the primary analysis, none of the baseline plasma biomarkers correlated with best overall response. Exploratory analyses showed that low VEGF-A levels were associated with longer progression-free survival (7.4 versus 6.1 months; hazard ratio, 1.57; 95% confidence intervals, 1.17 to 2.09; p = 0.002) and overall survival (19.8 versus 11.1 months; hazard ratio, 1.57; 95% confidence interval, 1.15–2.13; p = 0.004) compared with these in high baseline plasma VEGF-A levels. No plasma biomarkers changed significantly over time. No significant correlations were observed between tumor biomarkers and clinical outcomes. No new safety signals were observed.

Conclusion: Baseline and/or dynamic changes in plasma basic fibroblast growth factor, E-selectin, intercellular adhesion molecule-1, placental growth factor, VEGFR-1 and VEGFR-2, and tumor biomarkers did not correlate statistically with treatment outcomes for bevacizumab plus chemotherapy. Only baseline plasma VEGF-A was significantly correlated with progression-free survival/overall survival.

Key Words: Non–small-cell lung cancer, Biomarker, Bevacizumab, Vascular endothelial growth factor.

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Patients with advanced-stage non–small-cell lung cancer (NSCLC) typically receive first-line platinum-based chemotherapy, with a median overall survival (OS) consistently less than 12 months.[1,2] NSCLC is a heterogeneous disease of several histological subtypes and molecular profiles, which may explain the limited efficacy of platinum-based chemotherapy in an unselected population. Multiple pathways play a role in the pathogenesis and progression of lung tumors; however, only mutations in the epidermal growth factor receptor and echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase fusions are currently used to guide therapy. Because only a proportion of NSCLC tumors harbor these oncogenic driver mutations, chemotherapy-based treatment has remained the standard for most patients without these known biomarkers.

*State Key Laboratory of Southern China, Hong Kong Cancer Institute, Department of Clinical Oncology, Prince of Wales Hospital, Hong Kong, China; †Russian Research Oncology Center n.a. N.N. Blokhin of the Russian Academy of Medical Sciences, Moscow, Russia; ‡Országos Korányi TBC és Pulmonológiai Intézet, Budapest, Hungary; §Department of Pulmonology, Markusovszky University Hospital, Budapest, Hungary; ‖Arkhangelsk Regional Clinical Oncology Dispensary, Perm, Russia; ¶Department of Internal Medicine, National Taiwan University Hospital, Taipei City, Taiwan; #Roche Products Limited, Welwyn Garden City, Hertfordshire, United Kingdom; **F. Hoffmann-La Roche Ltd., Basel, Switzerland; and ††Department of Thoracic Oncology, Lung Clinic Grosshansdorf, Member of the German Center for Lung Research (DZL), Grosshansdorf, Germany.

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Address for correspondence: Tony Mok, MD, Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong, China. E-mail: tony@clo.cuhk.edu.hk

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Bevacizumab is an antivascular endothelial growth factor (VEGF) monoclonal antibody with activity in multiple tumor types. In unselected patients with advanced nonsquamous NSCLC, first-line combination therapy with bevacizumab (15 mg/kg per 3-weekly) and carboplatin/paclitaxel demonstrated OS, progression-free survival (PFS), and objective response rate (ORR) improvements over chemotherapy alone.[3] PFS and ORR improvements were also reported for bevacizumab (7.5 or 15 mg/kg per 3-weekly) in combination with cisplatin/gemcitabine, compared with chemotherapy alone.[4] Currently, no validated biomarker for bevacizumab is known, but several candidate biomarkers have been examined. A correlation between plasma VEGF and ORR was demonstrated for bevacizumab in NSCLC[5] and other cancers (breast, colon, gastric, and pancreatic).[6–10] Other angiogenesis biomarkers, including E-selectin, intercellular adhesion molecule (ICAM-1), and fibroblast growth factor (FGF), have potential correlations with treatment outcome in patients with NSCLC.[5] Those markers were examined as part of the comprehensive biomarker research program implemented across multiple tumor types, which aimed to identify patients who may benefit most from receiving bevacizumab therapy.

Avastin Biomarkers In lunG And 3D Innovative anaLysis (ABIGAIL; BO21015) was the first study to prospectively investigate the correlation between plasma biomarkers and best overall response (BOR) to bevacizumab plus platinum-doublet chemotherapy for patients with advanced/recurrent NSCLC. The primary objective was to correlate BOR with baseline plasma levels of VEGF-A, VEGF receptors (VEGFR-1 and VEGFR-2), basic FGF (bFGF), E-selectin, ICAM-1, and placental growth factor (PlGF). In keeping with a European Union postapproval requirement, exploratory analyses of efficacy and safety for the 7.5 and 15 mg/kg 3-weekly doses of bevacizumab plus carboplatin-based chemotherapy were performed. This article describes the primary analyses (baseline plasma biomarker protein levels), secondary measures of efficacy and safety of the two bevacizumab doses, and the exploratory end points (changes in biomarker levels and correlative analyses of tumor markers).

PATIENTS AND METHODS

Study Design and Patients

ABIGAIL was an international, multicenter, open-label, randomized, phase II trial in chemonaive patients with advanced/recurrent nonsquamous NSCLC. Patients received bevacizumab plus carboplatin/gemcitabine or carboplatin/paclitaxel, at the investigator's discretion.

Eligible patients were randomized (centralized stratified assignment) to receive bevacizumab (Avastin®; F. Hoffmann-La Roche, Basel, Switzerland) 7.5 or 15 mg/kg plus chemotherapy. Stratification factors were chemotherapy regimen, disease stage (IIIB/IV/recurrent), sex, and Eastern Cooperative Oncology Group performance status. According to investigators' preference, chemotherapy was either carboplatin (area under the curve × 5) on day 1 plus gemcitabine 1200 mg/m^2 on days 1 and 8 or carboplatin (area under the curve × 6) on day 1 plus paclitaxel 200 mg/m^2 on day 1. The chemotherapy regimens were selected to allow compliance with the Sponsor's regulatory commitments and to allow for differences in clinical practice and patient comorbidities. Only two regimens were permitted to reduce the potential impact that different chemotherapy regimens might have on the primary biomarker end point. A balance of chemotherapy regimens and baseline patient characteristics between the treatment arms was important; therefore, chemotherapy regimen was included as a stratification factor for randomization. Chemotherapy was given for up to six cycles at 3-weekly intervals unless progressive disease (PD) or unacceptable toxicity was observed. Bevacizumab was administered concurrently with chemotherapy on day 1 of each cycle and continued as monotherapy until PD/unacceptable toxicity. Crossover from gemcitabine to paclitaxel and vice versa was not permitted.

Patients aged 18 years or older with histologically/cytologically confirmed stage IIIB/IV, chemonaive, metastatic, or recurrent nonsquamous NSCLC were enrolled between September 2008 and November 2009. Patients had Eastern Cooperative Oncology Group performance status 0/1 and measurable disease as assessed by Response Evaluation Criteria in Solid Tumors v1.0. Exclusion criteria included the following: prior chemotherapy with another systemic anticancer agent; clinical evidence of central nervous system metastases; history of grade 2 or more hemoptysis; evidence of tumor invading or abutting major blood vessels; malignancies other than NSCLC within 5 years before randomization other than adequately treated cancer in situ of the cervix, basal or squamous-cell skin cancer, localized prostate cancer, or ductal carcinoma in situ; clinically significant cardiovascular disease; uncontrolled hypertension; current or recent use of aspirin (>325 mg/day); or full-dose anticoagulants or thrombolytic agents.

The trial was approved by local independent ethics committees and was conducted in accordance with the principles of the Declaration of Helsinki and Guidelines of Good Clinical Practice. This study is registered with ClinicalTrials.gov (NCT00700180).

Biomarker Sample Collection

Samples were prospectively collected and analyzed according to protocol. Patients provided mandatory plasma samples (5 ml of blood in ethylenediaminetetraacetic acid) at: baseline; every second cycle during combination treatment; completion of the sixth cycle; every cycle for 4 months and thereafter every second cycle during bevacizumab monotherapy; and at PD. A mandatory baseline 3 ml whole-blood sample for clinical genotyping/DNA analysis was provided. Baseline tumor samples from initial diagnosis were collected (where available) as paraffin-embedded tissue blocks or 20 tissue sections or more as unstained, uncovered slides.

Biomarker Assessments and Methodology

The candidate plasma biomarkers were VEGF-A, VEGFR-1, VEGFR-2, bFGF, E-selectin, PlGF, and ICAM-1. Plasma samples (except PlGF) were analyzed centrally (Roche Diagnostics Ltd., Penzberg, Germany) using

immunological multiparametric chip technique technology, a Roche proprietary multiplex enzyme-linked immunosorbent assay platform. Plasma VEGF-A was measured at baseline only, as the assay does not reliably measure VEGF-A during bevacizumab treatment.

Plasma PlGF analysis was performed centrally (Covance Central Laboratory Services Inc., Indianapolis, IN) using enzyme-linked immunosorbent assay (Quantikine®; R&D Systems, Minneapolis, MN) and environmental impact assessment methodology on a Bio-Tek ELx800 automated microplate reader. Tumor tissue biomarkers (VEGF-A, VEGFR-1, VEGFR-2, NRP-1, and CD31 [microvessel density]) were analyzed centrally (Targos Molecular Pathology, Kassel, Germany). Immunohistochemistry was performed on 5-μm sections of paraffin-embedded tissue. To assess tissue biomarker expression, an H-score was calculated for each sample other than CD31.

Safety and Efficacy Assessment

Tumors were investigator-evaluated (according to Response Evaluation Criteria in Solid Tumors) at baseline and 6-weekly (i.e., every two cycles) during the trial. Patients who withdrew from the trial (excluding for PD) were assessed at 6-weekly intervals until PD. Tumor size was assessed using high-resolution computed tomography scans, obtained according to a prespecified acquisition protocol. An independent Data and Safety Monitoring Board was responsible for the ongoing review of unblinded safety data. Adverse events (AEs) were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 and coded according to the Medical Dictionary for Regulatory Activities (MedDRA v14.0).

Statistical Analyses

Analysis of the primary end point and all secondary end points, except OS, was performed using a data cutoff of September 17, 2010. OS was analyzed using a data cutoff of July 11, 2011.

A sample size of approximately 300 patients was chosen to provide sufficient data to generate biomarker hypotheses (no formal hypothesis testing was planned or performed). Assuming an ORR of approximately 30%, and on the basis of a type α (or type I) error of 0.01 (to account for multiple testing), the study had 80% power to detect an odds ratio of greater than 2.24 for the difference in BOR between high and low groups.

The treatment arms were pooled for the primary analysis, which predefined baseline plasma VEGF-A, VEGFR-1, VEGFR-2, bFGF, E-selectin, PlGF, and ICAM-1 as the primary set of biomarkers.

Plasma biomarkers were analyzed as continuous variables using a log2 transformation. Tumor biomarkers were analyzed as continuous variables but were not log transformed. Biomarkers were dichotomized: patient populations were divided into high (≥ median) or low (<median) groups for each analysis, using the sample median plasma concentration or the sample median tumor H-score as the cutoff. PFS was a secondary end point, and an exploratory equivalence boundary was calculated as follows: in the E4599 study, the median PFS in the 15 mg/kg bevacizumab arm was 6.4 months; therefore, with 15-month linear recruitment and 10-month minimum follow-up in 300 patients, approximately 249 events were expected. Under the assumption that the hazard ratio (HR) is 1 (i.e., the treatment arms have equivalent PFS) with 80% power and 95% confidence, the boundaries for equivalence were 0.70 and 1.43.

Correlation of Plasma and Tumor Markers with BOR/PFS/OS

Exploratory analyses of the correlations between biomarker levels and the following end points were carried out: BOR, PFS (randomization to PD/death), and OS (randomization to death, irrespective of cause). The biomarker odds ratio was tested using Wald's test. No formal adjustments were made for multiple testing. Correlation of biomarker levels with BOR was performed using logistic regression with correction for baseline prognostic factors. Multiple Cox regression models were run for PFS/OS using a similar strategy. Dynamic evaluation of plasma markers (change from baseline) were presented as box plots.

RESULTS

Patient Characteristics

Of 303 patients randomized (49 centers; 15 countries [Europe, Eastern Asia, Australia, and Canada]), 154 received bevacizumab 7.5 mg/kg plus chemotherapy (carboplatin/gemcitabine or carboplatin/paclitaxel) and 149 received bevacizumab 15 mg/kg plus chemotherapy (Fig. 1). Table 1 lists the baseline characteristics of the intention-to-treat (ITT), biomarker-evaluable plasma, and biomarker-evaluable immunohistochemistry populations. Generally, the biomarker-evaluable plasma and biomarker-evaluable immunohistochemistry baseline characteristics were balanced between arms.

Primary Analysis: Correlation of Baseline Plasma Biomarker Level with BOR

Baseline plasma level did not correlate with response for any of the seven markers assessed. Table 2 summarizes the BOR in patients with low and high baseline biomarker levels. The *p* value for E-selectin was less than 0.05 but not statistically significant after adjustment for multiple testing.

Exploratory Analyses

Correlation of baseline plasma markers with efficacy parameters

Only VEGF-A at baseline seemed to significantly correlate with PFS and OS (Fig. 2). Low baseline VEGF-A levels were associated with longer PFS (7.4 months) versus high baseline VEGF-A (6.1 months): HR, 1.57 (95% confidence interval [CI], 1.17 to 2.09); $p = 0.002$. Similarly, low baseline VEGF-A levels were associated with longer median OS (19.8 months) compared with that in high baseline VEGF-A levels (11.1 months; HR, 1.57; 95% CI, 1.15 to

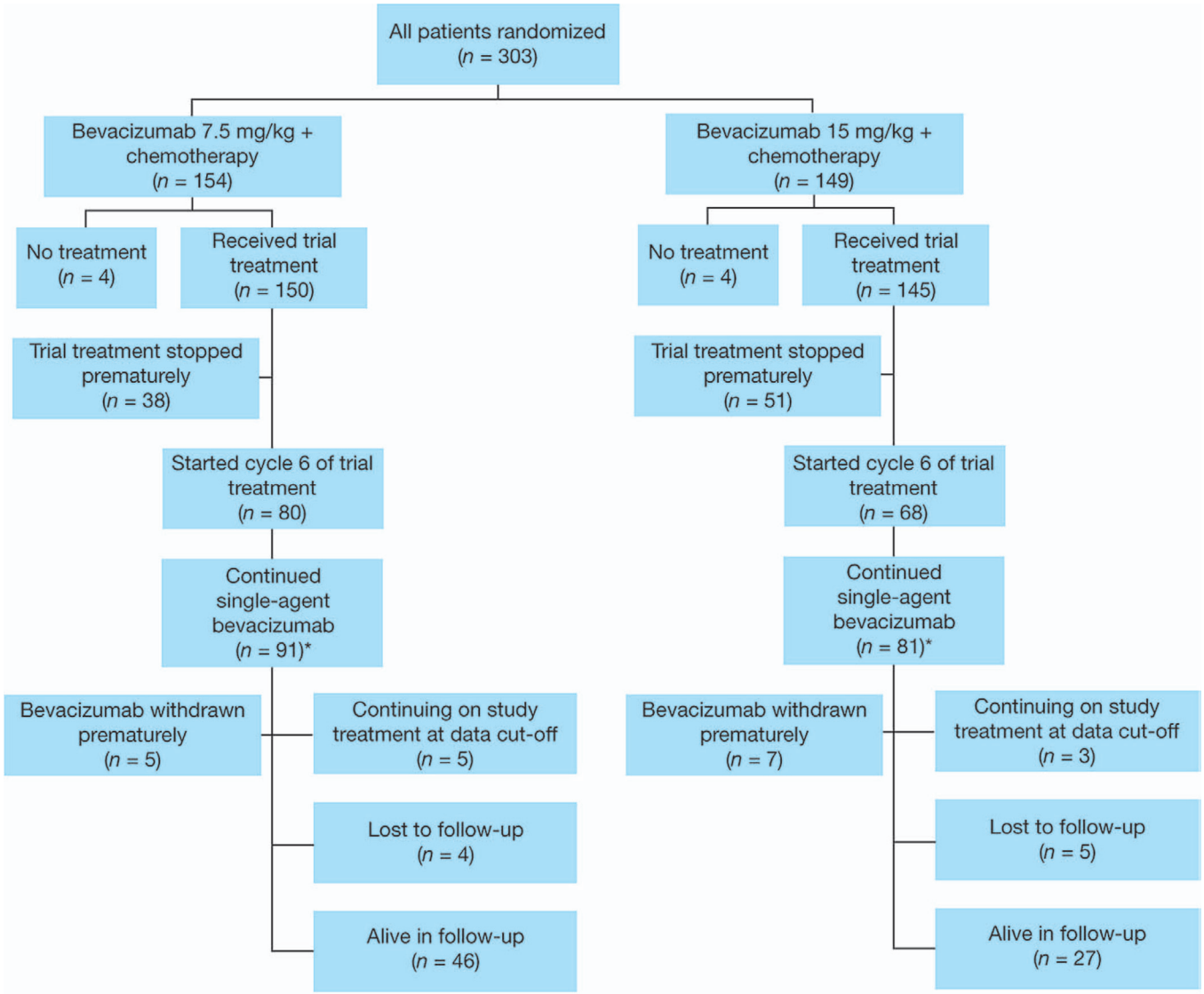

FIGURE 1. Patient disposition during the study. *Includes 11 patients receiving bevacizumab 7.5 mg/kg plus chemotherapy and 13 patients receiving bevacizumab 15 mg/kg plus chemotherapy who had prematurely withdrawn from at least one component of chemotherapy but had continued bevacizumab. In the bevacizumab 7.5 mg/kg + chemotherapy arm, five patients had bevacizumab withdrawn prematurely, four were lost to follow-up, 46 were alive in follow-up, and five were continuing on study treatment at data cutoff. In the bevacizumab 15 mg/kg + chemotherapy arm, seven patients had bevacizumab withdrawn prematurely, five were lost to follow-up, 27 were alive in follow-up, and three were continuing on study treatment at data cutoff.

2.13]; $p = 0.004$). Considering a total of seven tests, adjusting for multiple testing would still result in a statistically significant p value.

Exploration of change from baseline plasma marker levels

At least one postbaseline plasma sample was available from approximately 74% of patients for each of the candidate biomarkers. Plasma biomarker levels for bFGF, E-selectin, ICAM-1, VEGFR-1, and VEGFR-2 were not different between baseline and time of progression (Supplementary Fig. 1, Supplementary Digital Content 1, http://links.lww.com/JTO/A564) and were not different between baseline and cycles two, four, or six for any of the biomarkers tested.

Correlation of tumor protein markers with efficacy parameters

The percentage of patients with available tumor samples for analysis for each biomarker ranged from 19.5% to 30.7% (Supplementary Table 1, Supplementary Digital Content 2, http://links.lww.com/JTO/A565). There seemed to be an association between tumor VEGFR-1 and treatment effect. High tumor levels of VEGFR-1 were associated with shorter OS ($p = 0.0371$), but this was not statistically significant after

TABLE 1. Baseline Characteristics of the ITT, BEP, and BEI Populations

	ITT		BEP		BEI	
Characteristic, *n* (%)	Bev 7.5 mg + Chemo (*n* = 154)	Bev 15 mg + Chemo (*n* = 149)	Bev 7.5 mg + Chemo (*n* = 144)	Bev 15 mg + Chemo (*n* =143)	Bev 7.5 mg + Chemo (*n* = 44)	Bev 15 mg + Chemo (*n* = 50)
Sex						
Female	56 (36)	55 (37)	53 (37)	55 (38)	12 (27)	20 (40)
Male	98 (64)	94 (63)	91 (63)	88 (62)	32 (73)	30 (60)
Ethnicity						
Caucasian	131 (85)	127 (85)	122 (85)	122 (85)	36 (82)	44 (88)
Asian	23 (15)	21 (14)	22 (15)	20 (14)	8 (18)	5 (10)
Other	0 (0)	1 (<1)	0 (0)	1 (<1)	0 (0)	1 (2)
Smoking status						
Never smoker	49 (32)	40 (27)	49 (34)	38 (27)	14 (32)	13 (26)
Former smoker	60 (39)	70 (47)	54 (38)	68 (48)	16 (36)	23 (46)
Current smoker	44 (29)	39 (26)	40 (28)	37 (26)	14 (32)	14 (28)
ECOG PS						
0	56 (36)	53 (36)	52 (36)	49 (34)	16 (36)	17 (34)
1	98 (64)	96 (64)	92 (64)	94 (66)	28 (64)	33 (66)

BEI, biomarker-evaluable immunohistochemistry; BEP, biomarker-evaluable plasma; ECOG PS, Eastern Cooperative Oncology Group performance status; ITT, intention-to-treat.

TABLE 2. Summary of BOR, PFS, and OS by Baseline Plasma Biomarkers

	Low BM Level		High BM Level		Logistic Regression		
BOR	***n***	**Responders, %**	***n***	**Responders, %**	**OR***	**95% CI**	***p* Value**
bFGF	142	45.07	141	42.55	1.07	0.63–1.80	0.8127
E-selectin	142	39.44	141	48.23	1.81	1.06–3.08	0.0285
ICAM	142	44.37	141	43.26	1.09	0.64–1.85	0.7478
PlGF	146	43.87	56	42.86	1.16	0.58–2.33	0.6761
VEGF-A	140	43.57	140	45.00	1.22	0.72–2.09	0.4601
VEGFR-1	142	48.59	141	39.01	0.77	0.46–1.29	0.3193
VEGFR-2	143	39.16	140	48.57	1.44	0.85–2.45	0.1758

	Low BM Level			High BM Level			Cox Regression		
PFS	***n***	**Events, *n* (%)**	**Median PFS, Months**	***n***	**Events, *n* (%)**	**Median PFS, Months**	**HR***	**95% CI**	***p* Value**
bFGF	142	116 (82)	7.2	141	124 (88)	6.5	1.21	0.92–1.59	0.170
E-selectin	142	119 (84)	6.6	141	121 (86)	6.8	0.94	0.72–1.24	0.684
ICAM	142	118 (83)	7.0	141	122 (87)	6.3	1.18	0.89–1.56	0.250
PlGF	146	122 (84)	6.7	56	51 (91)	6.3	1.20	0.85–1.71	0.308
VEGF-A	140	111 (79)	7.4	140	126 (90)	6.1	1.57	1.17–2.09	0.002
VEGFR-1	142	119 (84)	7.2	141	121 (86)	6.2	1.14	0.87–1.49	0.351
VEGFR-2	143	120 (84)	6.7	140	120 (86)	6.9	0.95	0.72–1.26	0.724

OS	***n***	**Events, *n* (%)**	**Median OS, Months**	***n***	**Events, *n* (%)**	**Median OS, Months**	**HR***	**95% CI**	***p* Value**
bFGF	142	94 (66)	17.0	141	105 (75)	13.1	1.11	0.82–1.50	0.5101
E-selectin	142	97 (68)	13.7	141	102 (72)	14.0	0.91	0.67–1.22	0.5176
ICAM	142	91 (64)	16.4	141	108 (77)	12.3	1.23	0.90–1.67	0.1934
PlGF	146	97 (66)	17.0	56	42 (75)	11.4	1.11	0.75–1.64	0.6035
VEGF-A	140	83 (59)	19.8	140	113 (81)	11.1	1.57	1.15–2.13	0.0042
VEGFR-1	142	94 (66)	17.4	141	105 (75)	11.5	1.30	0.97–1.75	0.0827
VEGFR-2	143	100 (70)	13.4	140	99 (71)	14.2	0.83	0.61–1.13	0.2435

*OR/HR: high vs. low BM level.

bFGF, basic fibroblast growth factor; BM, blood glucose monitoring; BOR, best overall response; CI, confidence interval; HR, hazard ratio; ICAM, intercellular adhesion molecule; OR, odds ratio; OS, overall survival; PFS, progression-free survival; PlGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

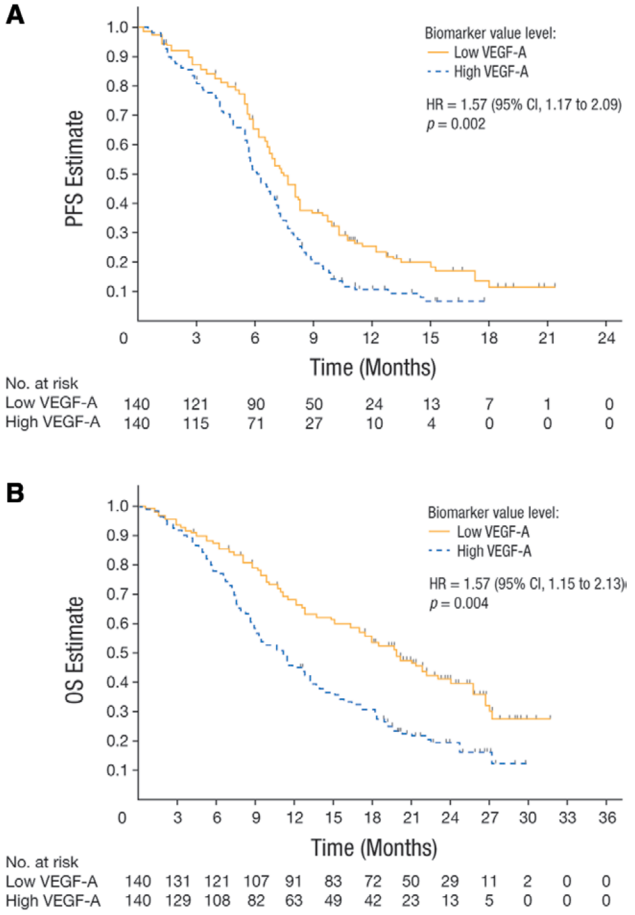

FIGURE 2. *A*, PFS and (*B*) OS relative to baseline plasma VEGF-A levels. CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; VEGF, vascular endothelial growth factor.

adjustment for multiple testing. No statistically significant correlations were observed between the tumor markers investigated and BOR, PFS, or OS.

Correlation between tumor and plasma markers

An exploration of the correlation between patients' baseline tumor VEGFR-1 expression and baseline VEGF-A plasma level revealed a possible correlation between the two (0.26, $p = 0.025$) (Supplementary Fig. 2, Supplementary Digital Content 3, http://links.lww.com/JTO/A566).

Efficacy in the Overall Population

ORR was 37.1% (7.5 mg/kg bevacizumab) versus 46.4% (15 mg/kg bevacizumab) ($p = 0.1737$). Disease control rate was similar between arms at 76.8% (7.5 mg/kg) and 78.5% (15 mg/kg; $p = 0.6148$). PFS was similar in the ITT population between doses: 6.8 months (7.5 mg/kg) and 6.7 months (15 mg/kg): HR, 1.01; 95% CI, 0.78 to 1.31; as noted, 95% CI equivalence boundaries of 0.70 and 1.43 were defined. Supplementary Figure 3 (Supplementary Digital Content 4, http://links.lww.com/JTO/A567) shows subgroup analyses of PFS according to bevacizumab dose/chemotherapy regimen. Similar results to the ITT analysis were apparent in all four subgroups; however, this comparison was nonrandomized and patient numbers were small. Median OS also seemed similar across treatment arms (13.4 and 13.7 months for the 7.5 and 15 mg/kg arms, respectively; HR, 1.16; 95% CI, 0.87–1.53) although the trial was not powered for OS.

Subsequent Systemic Antineoplastic Therapy

Second-line therapy was given to 56% and 52% of patients (7.5 and 15 mg/kg arms, respectively) and the most common regimens were pemetrexed (16% versus 13%), erlotinib (12% versus 14%), gefitinib (6%, both arms), and docetaxel (5%, both arms). A total of 18% and 10% of patients (7.5 and 15 mg/kg arms, respectively) received third-line therapy, most often erlotinib (9% versus 4%) and pemetrexed (5% versus 2%).

Safety

All randomized patients who received at least one dose of trial treatment were included in the safety analysis. The median dose intensity was approximately 95% for bevacizumab, carboplatin, and paclitaxel and 89% for gemcitabine.

The most common AEs were predominantly grade 1/2. No new safety signals were observed compared with previous NSCLC bevacizumab studies. The incidence of the most common grade 3 or greater AEs was broadly similar across the two arms, with some exceptions (Supplementary Table 2, Supplementary Digital Content 5, http://links.lww.com/JTO/A568). A higher incidence of pulmonary embolism was observed in the 15 mg/kg bevacizumab arm versus the 7.5 mg/kg arm, whereas the 7.5 mg/kg arm had higher incidences of neutropenia, thrombocytopenia, and leukopenia. AEs of special interest for bevacizumab are also summarized (Supplementary Table 2, Supplementary Digital Content 5, http://links.lww.com/JTO/A568). More bleeding events were observed with 7.5 mg/kg bevacizumab than with the higher dose. One patient with squamous-cell carcinoma who was erroneously randomized into the study (7.5 mg/kg arm) had a fatal pulmonary hemorrhage. The incidence of venous thromboembolism was higher in the 15 mg/kg group. The most common cause of death was disease progression.

DISCUSSION

ABIGAIL was the first prospective study on the correlation between plasma biomarkers and tumor response in advanced NSCLC. None of the angiogenic plasma biomarkers investigated correlated with tumor response to chemotherapy plus bevacizumab. Only low baseline plasma VEGF-A level was correlated with longer PFS and OS. VEGF-A is the primary ligand targeted by bevacizumab and, in the absence of a control arm, the observed correlation could be explained by a prognostic and/or predictive role for the biomarker. The data reported here do not contradict previous studies, which report potential prognostic and/or predictive association of VEGF-A with clinical outcomes in bevacizumab-treated NSCLC and other tumor types.

Several studies have reported a potential prognostic value of VEGF in NSCLC at tumor level.[11–21] Although

fewer studies have investigated the role of circulating VEGF levels as prognostic markers of patient outcomes in NSCLC and other tumor types,[6,8,22,23] and although the data are more heterogeneous, the evidence is compelling. Further investigations are needed to validate these results.

The value of VEGF-A as a potential predictive marker for clinical outcomes with bevacizumab was also investigated. High plasma VEGF-A levels were predictive of increased response to bevacizumab plus carboplatin/paclitaxel compared with carboplatin/paclitaxel alone in NSCLC patients in the E4599 study.[5] In contrast, the predictive value of VEGF-A was not observed for OS in the Avastin in Lung (AVAiL) study of patients with NSCLC randomized to platinum-based chemotherapy with or without bevacizumab.[6,24] Plasma VEGF-A levels have shown potential predictive value in trials of bevacizumab in other tumor types. Biomarker analyses from the Avastin and Docetaxel (AVADO), Avastin in Gastric Cancer (AVAGAST), and Avastin and Tarceva in Advanced Pancreatic Cancer (AVITA) studies of bevacizumab plus chemotherapy for the first-line treatment of human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer, advanced gastric cancer, and metastatic pancreatic cancer, respectively, showed that high plasma VEGF-A levels correlated with improved outcomes after bevacizumab treatment, indicating a possible predictive value.[7,9,10] Interpretation of these apparently different results is complex. Although true-negative results in NSCLC cannot be excluded, confounding factors such as sample preparation and analytical variability may have contributed to the conflicting intertrial findings.

Differences in analytical sensitivity may have been a factor in the apparently varying predictive value of VEGF-A across tumor types. The presence of shorter isoforms (VEGF-A121 and VEGF-A110), which are detected with greater sensitivity than longer isoforms by the immunological multiparametric chip technique assay, may vary between tumor types and contribute to heterogeneity of predictive value across tumor type. The isoform VEGF-A189, for example, was more frequently expressed in NSCLC (90.5%) than in extraneoplastic lung tissue (57.6%, $p = 0.00004$).[21]

Controversy remains regarding the question of whether plasma, serum, or whole blood provides the best representation of the tumor site.[25–27] Patients with more than two metastatic tumor sites or more advanced stage of disease (stage IV versus IIIB) tended to have higher baseline levels of plasma VEGF-A, suggesting that VEGF-A level may reflect tumor volume. It is important in the era of personalized medicine that clinicians reach a consensus on the preferred biospecimen and analytical methodology to facilitate accurate data interpretation and enable cross-trial comparisons.

Other baseline plasma markers including ICAM-1, bFGF, VEGFR-1, VEGFR-2, PlGF, and E-selectin were not correlated with tumor response rate or survival. This is consistent with a retrospective biomarker analysis of the E4599 study, which reported similar findings.[5] Furthermore, plasma biomarker levels did not change significantly over time (as assessed by serial measurements at cycles two, four, and six), thus dynamic change would have minimal predictive value. The results of the ABIGAIL study suggest that further investigation of any of these single biomarkers for bevacizumab is not warranted.

The secondary efficacy outcomes of ABIGAIL were similar to those in the pivotal phase III E4599 and AVAiL studies.[3,4] Median OS point estimates were comparable with AVAiL and E4599 (12.3–13.6 months), and the median PFS in ABIGAIL was similar to the median PFS for the bevacizumab group in the E4599 study (6.2 months). Efficacy outcomes for bevacizumab at the two dose levels (7.5 and 15 mg/kg) were similar in the ABIGAIL study, but the limited sample size and exploratory nature of the study mean that it is not possible to draw formal statistical conclusions regarding the doses. With regard to safety, more hemorrhagic events were associated with the 7.5 mg/kg dose and more thromboembolic events with the 15 mg/kg dose. Three grade 5 hemorrhage events occurred, one of which was associated with a protocol violation. The overall incidence of bevacizumab-associated toxicity in ABIGAIL was similar to E4599 and AVAiL.[3,4] The safety of the 7.5 and 15 mg/kg treatment arms seemed similar.

In summary, baseline and dynamic change in plasma levels of bFGF, E-selectin, ICAM-1, PlGF, VEGFR-1, and VEGFR-2 did not correlate with tumor response for bevacizumab plus chemotherapy. Low baseline plasma levels of VEGF-A had a statistically significant correlation with longer PFS and OS. VEGF-A may be a promising biomarker, but the current study cannot determine the nature of the observed effect, that is, whether it is predictive or prognostic, due to the lack of a control arm. Future randomized studies would be needed to further explore the potential predictive value of plasma VEGF-A.

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